

EXHIBIT B20

Part 1

Talc / Baby Powder

10/15/17

Tried to dissolve talc (Fisher # T4-500 Lot #166820) or
 Johnson+Johnson Baby Powder (# 30027477, Lot 13717RA)

- It won't completely dissolve - used DMSO & filtered

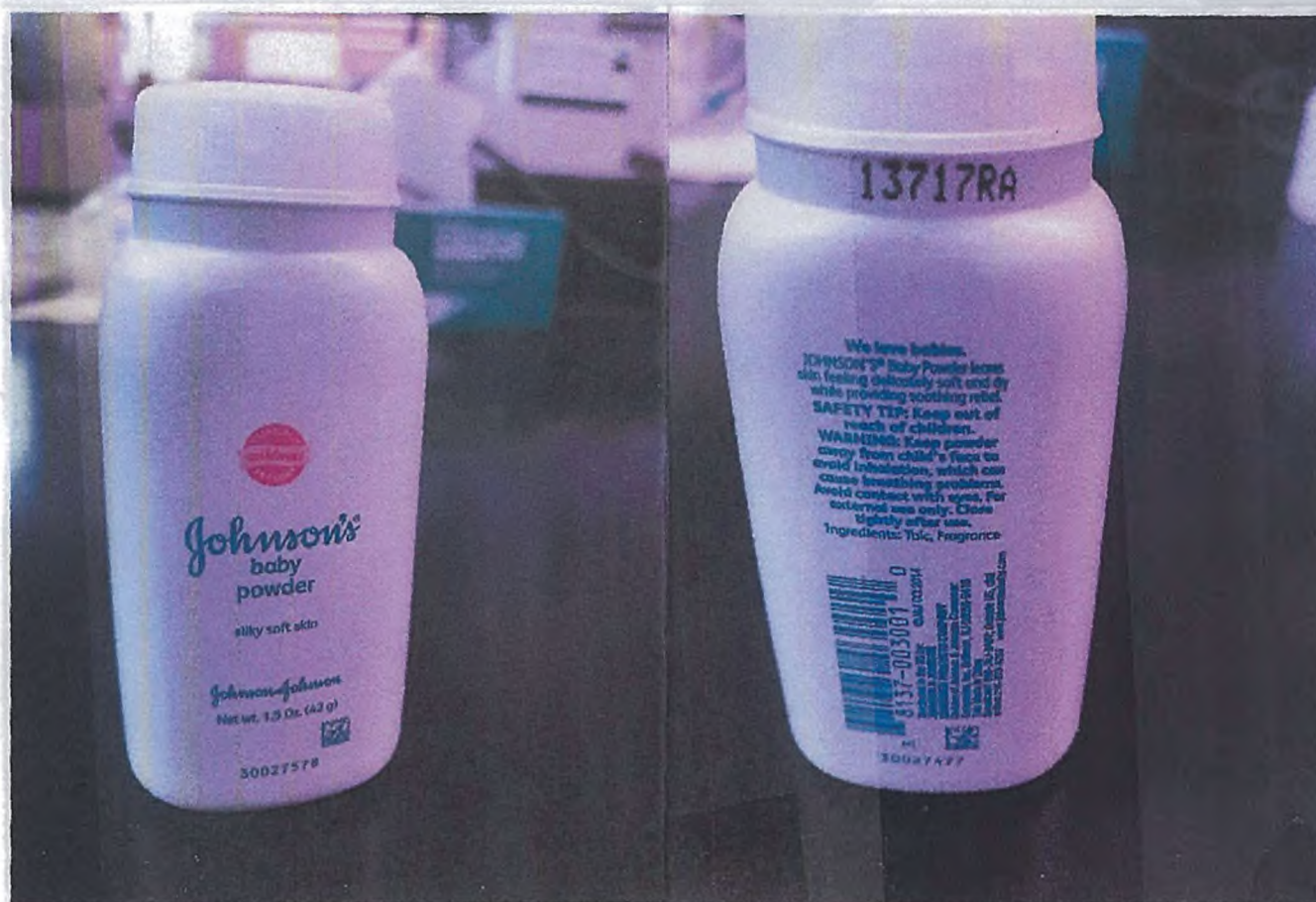
50mg/ml

treated 200 μ g/ml or 500 μ g/ml in dishes

8 μ l for 200 μ g/ml

20 μ l for 500 μ g/ml





11/13/17

Talc / Baby Powder Treated
EOC, Normal Ov. Epi, Macrophages

- Split OV90 cells, 150mm dish x2
- Split macrophages (had 4.8×10^6 cells in 150mm plate)
- Split CB. Normal Ov. Epi. cells
 - had 2×150 mm dish $\Rightarrow \sim 11 \times 10^6$ cells
 - split into 4 plates
- Seeded TOV112-D unt. cells - treat after 24hr rest -
(full plate was $\sim 12 \times 10^6$ cells)

6 plates per timepoint in 100mm dish

ID	treatment	details
266	TOV112 - unt-24hr	2×10^6 cells
267	Control (DMSO) -24h	"
268	500 μ g/ml Talc -24h	"
269	1000 μ g/ml Talc -24h	"
270	500 μ g/ml Baby Powder (BP) -24h	"
271	1000 μ g/ml BP -24h	"
272	TOV112 - unt 48hr	1×10^6 cells
273	Control 48h	"
274	500 μ g/ml Talc 48h	"
275	1000 μ g/ml Talc 48h	"
276	500 μ g/ml B.P. 48h	"
277	1000 μ g/ml B.P. 48h	"
278	TOV1120 - unt 72hr	500,000 cells
279	Control - 72hr	"
280	500 μ g/ml Talc -72h	"
281	1000 μ g/ml Talc -72h	"
282	500 μ g/ml B.P. 72h	"
283	1000 μ g/ml B.P. 72hr	"

from pg 2

11/14/17

Treat cells after 24 hr rest

$$(x)(50,000 \mu\text{g/ml}) = (10 \text{ ml})(500 \mu\text{g/ml})$$

$$x = 100 \mu\text{l}$$

$$\text{for } 1000 \mu\text{g/ml} = 200 \mu\text{l}$$

Controls get 200 μl of sterile DMSO

- made a master mix of media + treatment and then added it to the cells

$$500 \mu\text{g/ml} = 300 \mu\text{l} + 30 \text{ ml}$$

$$1000 \mu\text{g/ml} = 600 \mu\text{l} + 30 \text{ ml}$$

$$\text{DMSO Control} = 600 \mu\text{l} + 30 \text{ ml}$$

ID	Treatment
284	OV90 Untreated 24 hours
285	24 hr DMSO Control
286	24 hr 500 ug/ml Talc
287	25 hr 1000 ug/ml Talc
288	24 hr 500 ug/ml Baby Powder
289	24 hr 1000 ug/ml Baby Powder
290	Ov90 Untreated 48 hours
291	48 hr DMSO Control
292	48 hr 500 ug/ml Talc
293	48 hr 1000 ug/ml Talc
294	48 hr 500 ug/ml Baby Powder
295	48 hr 1000 ug/ml Baby Powder
296	Ov90 72 hr untreated
297	72 hr DMSO Control
298	72 hr 500 ug/ml Talc
299	72 hr 1000 ug/ml Talc
300	72 hr 500 ug/ml Baby Powder
301	72 hr 1000 ug/ml Baby Powder
302	EL1 Untreated 24 hours
303	24 hr DMSO Control
304	24 hr 500 ug/ml Talc
305	25 hr 1000 ug/ml Talc
306	24 hr 500 ug/ml Baby Powder
307	24 hr 1000 ug/ml Baby Powder
308	EL1 Untreated 48 hours
309	48 hr DMSO Control
310	48 hr 500 ug/ml Talc
311	48 hr 1000 ug/ml Talc
312	48 hr 500 ug/ml Baby Powder
313	48 hr 1000 ug/ml Baby Powder
314	EL1 72 hr untreated
315	72 hr DMSO Control
316	72 hr 500 ug/ml Talc
317	72 hr 1000 ug/ml Talc
318	72 hr 500 ug/ml Baby Powder
319	72 hr 1000 ug/ml Baby Powder

ID	Treatment
320	TOV-21G Untreated 24 hours
321	24 hr DMSO Control
322	24 hr 500 ug/ml Talc
323	25 hr 1000 ug/ml Talc
324	24 hr 500 ug/ml Baby Powder
325	24 hr 1000 ug/ml Baby Powder
326	TOV-21G Untreated 48 hours
327	48 hr DMSO Control
328	48 hr 500 ug/ml Talc
329	48 hr 1000 ug/ml Talc
330	48 hr 500 ug/ml Baby Powder
331	48 hr 1000 ug/ml Baby Powder
332	TOV-21G 72 hr untreated
333	72 hr DMSO Control
334	72 hr 500 ug/ml Talc
335	72 hr 1000 ug/ml Talc
336	72 hr 500 ug/ml Baby Powder
337	72 hr 1000 ug/ml Baby Powder
338	Cell Biologics - Normal Ovarian Epithelial cells, Unt 24 hrs
339	24 hr DMSO Control
340	24 hr 500 ug/ml Talc
341	25 hr 1000 ug/ml Talc
342	24 hr 500 ug/ml Baby Powder
343	24 hr 1000 ug/ml Baby Powder
344	Cell Biologics - Normal Ovarian Epithelial cells, Unt 48 hrs
345	48 hr DMSO Control
346	48 hr 500 ug/ml Talc
347	48 hr 1000 ug/ml Talc
348	48 hr 500 ug/ml Baby Powder
349	48 hr 1000 ug/ml Baby Powder
350	Cell Biologics - Normal Ovarian Epithelial cells, Unt 72 hrs
351	72 hr DMSO Control
352	72 hr 500 ug/ml Talc
353	72 hr 1000 ug/ml Talc
354	72 hr 500 ug/ml Baby Powder
355	72 hr 1000 ug/ml Baby Powder

11/17/17

seeded C.B. Normal Ov. Epi cells

338-355 - passage 11

24h = 2×10^6 cells48h = 1×10^6 cells

72h = 500,000 cells

Will treat on 11/19/17

collected 24hr 11/20/17

48hr 11/21/17

72h 11/22/17

11/19/17 seeded EL-1, treated on 11/19/17

for 24hr 4×10^6 collect 11/20/1748hr 2×10^6 collect 11/21/1772hr 1×10^6 collect 11/22/17

12/4/17 Seeded TON20 / TON21 G1 / treat 12/5/17

collect 24hr 12/6/17

" 48hr 12/7/17

" 72hr 12/8/17

12/7/17

RNA extraction

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
1	267	12/7/2017 2:07:20 PM	0.0635	µg/µl	1.588	0.831	1.91	1.36
2	269	12/7/2017 2:07:45 PM	0.0548	µg/µl	1.370	0.695	1.97	0.29
3	273	12/7/2017 2:08:08 PM	0.0257	µg/µl	0.643	0.335	1.92	0.24
4	275	12/7/2017 2:08:29 PM	0.0164	µg/µl	0.409	0.212	1.93	0.66
5	285	12/7/2017 2:08:53 PM	0.0678	µg/µl	1.694	0.882	1.92	1.13
6	287	12/7/2017 2:09:13 PM	0.0553	µg/µl	1.381	0.722	1.91	2.32
7	291	12/7/2017 2:09:32 PM	0.0630	µg/µl	1.575	0.802	1.96	0.34
8	293	12/7/2017 2:09:51 PM	0.0506	µg/µl	1.265	0.648	1.95	1.39
9	297	12/7/2017 2:10:10 PM	0.0358	µg/µl	0.896	0.455	1.97	0.22
10	299	12/7/2017 2:10:30 PM	0.0248	µg/µl	0.621	0.313	1.99	0.86
11	303	12/7/2017 2:10:51 PM	0.1809	µg/µl	4.523	2.334	1.94	1.35
12	305	12/7/2017 2:11:10 PM	0.1508	µg/µl	3.770	1.925	1.96	1.75
13	309	12/7/2017 2:11:31 PM	0.0279	µg/µl	0.698	0.362	1.93	0.85
14	311	12/7/2017 2:11:53 PM	0.0675	µg/µl	1.688	0.877	1.92	0.35
15	315	12/7/2017 2:12:12 PM	0.0445	µg/µl	1.113	0.585	1.90	1.13
16	317	12/7/2017 2:12:31 PM	0.0587	µg/µl	1.468	0.770	1.91	0.60
17	321	12/7/2017 2:12:50 PM	0.0810	µg/µl	2.025	1.061	1.91	1.03
18	323	12/7/2017 2:13:10 PM	0.0326	µg/µl	0.815	0.408	2.00	1.00
19	327	12/7/2017 2:13:31 PM	0.0445	µg/µl	1.112	0.574	1.94	2.54
20	329	12/7/2017 2:14:02 PM	0.0092	µg/µl	0.230	0.114	2.02	0.10
21	339	12/7/2017 2:14:21 PM	0.0177	µg/µl	0.442	0.220	2.01	0.55
22	341	12/7/2017 2:14:40 PM	0.0172	µg/µl	0.429	0.221	1.94	0.89
23	345	12/7/2017 2:14:59 PM	0.0219	µg/µl	0.548	0.281	1.95	1.31
24	347	12/7/2017 2:15:17 PM	0.0165	µg/µl	0.414	0.207	2.00	0.56
25	351	12/7/2017 2:15:34 PM	0.0165	µg/µl	0.413	0.214	1.93	0.96
26	353	12/7/2017 2:15:52 PM	0.0112	µg/µl	0.281	0.142	1.98	0.94
27	279	12/8/2017 1:19:05 PM	0.0145	µg/µl	0.364	0.192	1.89	1.07
28	281	12/8/2017 1:19:28 PM	0.0089	µg/µl	0.222	0.111	2.00	0.48
29	333	12/8/2017 1:19:56 PM	0.0244	µg/µl	0.609	0.317	1.92	0.65
30	335	12/8/2017 1:20:15 PM	0.0039	µg/µl	0.097	0.054	1.79	0.39
31	335	12/8/2017 1:21:01 PM	0.0041	µg/µl	0.102	0.048	2.12	0.38

12/8/17

(VILLO) CONA SYNTHESIS
0.1 μ g RNA used except
for #335 (0.06 μ g)

Sample ID	ul RNA for 0.1 μ g rxn	ul Water
267	1.6	14.4
269	1.8	14.2
273	3.9	12.1
275	6.1	9.9
279	6.9	9.1
281	11.2	4.8
285	1.5	14.5
287	1.8	14.2
291	1.6	14.4
293	2.0	14.0
297	2.8	13.2
299	4.0	12.0
303	0.6	15.4
305	0.7	15.3
309	3.6	12.4
311	1.5	14.5
315	2.2	13.8
317	1.7	14.3
321	1.2	14.8
323	3.1	12.9
327	2.2	13.8
329	10.9	5.1
333	4.1	11.9
335	16.0	0.0
339	5.6	10.4
341	5.8	10.2
345	4.6	11.4
347	6.1	9.9
351	6.1	9.9
353	8.9	7.1

1/10/18

CA-125 EUSA

RayBio # EHU-CA125

Test unconcentrated media vs concentrated using Amecim
Ultra-15 filter MW cutoff 10000

Weight tubes	Volume empty	empty sample reservoir w/ media	empty centrifuge tube	media + reservoir
266	8.36 mL 25.0 g	10.90 g	11.59 g	19.17 g
338	8.853 mL 24.9 g	10.98 g	11.53 g	19.7 g

Spin tubes for 25 min at 4000xg

move the retentate by pipetting into new container

weight retentate	tube weight	tube + retentate	filtrate
266	1.0025 g	1.3514 g	19.29 g
338	1.0088 g	1.7104 g	19.4797 g

$$\% \text{ retentate recovery} = 100 \times \frac{W_r \times C_r}{W_o \times C_o}$$

$$\% \text{ filtrate recovery} = 100 \times \frac{W_f \times C_f}{W_o \times C_o}$$

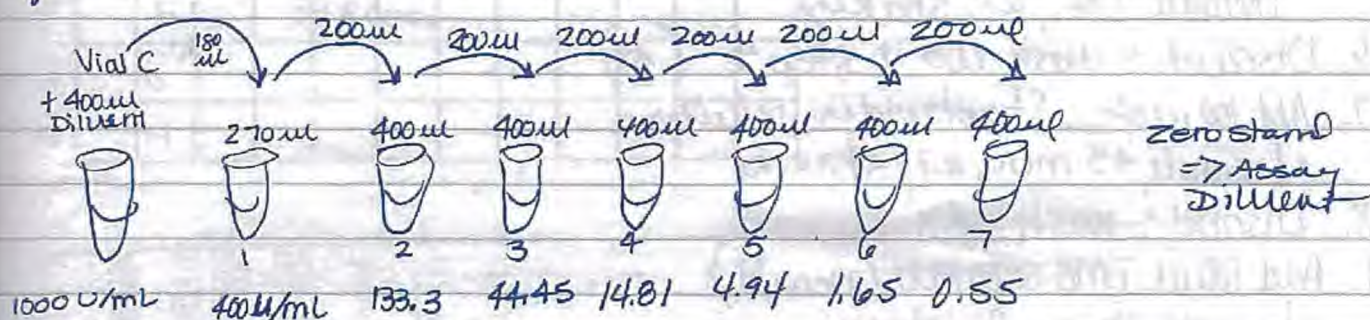
$$\% \text{ recovery} = \% \text{ retentate recovery} + \% \text{ filtrate recovery}$$

1/10/18

CA-125 EUSA

41040

1. All reagents & samples to room temperature
2. Assay diluent (Item E2) should be diluted 5x w/ dd H₂O
 - Stable 1 mo. at 4°C
3. Prep. of standard
 - Spin vial C
 - Add 400 μ l 1x Assay Diluent into vial C = 1000 u/mL
 - mix gently



4. Prepare Wash buffer by diluting 20x
 - Stable 1 mo. at 4°C

5. Spin Item F, detection antibody
 - Add 100 μ l of 1x Assay Diluent
 - Stable 5 days at 4°C
 - Dilute it ~~80x~~^{80x} and will be used in assay
 (1x Assay diluent)

6. Spin HRP-strep. Vial (Item G) & mix
 - Add 15 μ l to tube w/ 12 ml of assay diluent (800x)
 - do not save!

Go to pg 10

Form 991

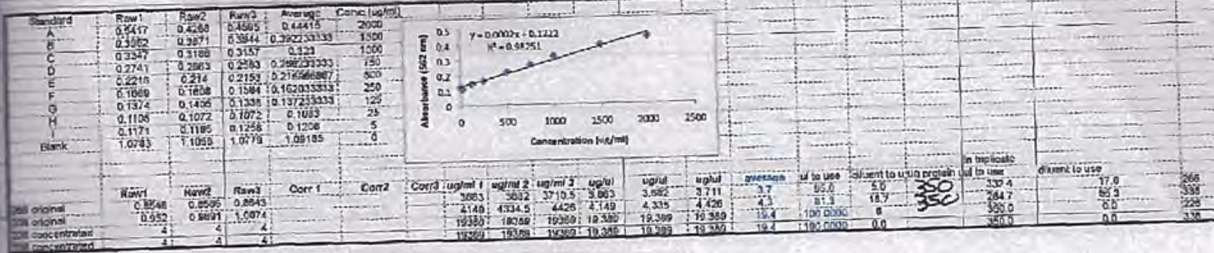
Assay Procedure

1. All samples to RT.
2. Label 8 strip/wells
3. Add 100 μ l standard / samples
 - Incubate 2.5 hr, gentle shaking, RT
4. Discard solution, wash 4x
 - decant, blot after washes
5. Add 100 μ l of 1x Antibody mix
 - Incubate 1 hr, RT, shaking
6. Discard & wash as in step 4 (4x)
7. Add 100 μ l of Streptavidin solution
 - Incubate 45 min, RT, shaking
8. Discard & wash 4x
9. Add 100 μ l TMB substrate (Item H)
 - Incubate 30 min, RT, shaking
10. Add 50 μ l STOP solution (Item I) to each well
 - Read at 450nm

1/10/18

Protein levels in media for
CA125 Assay

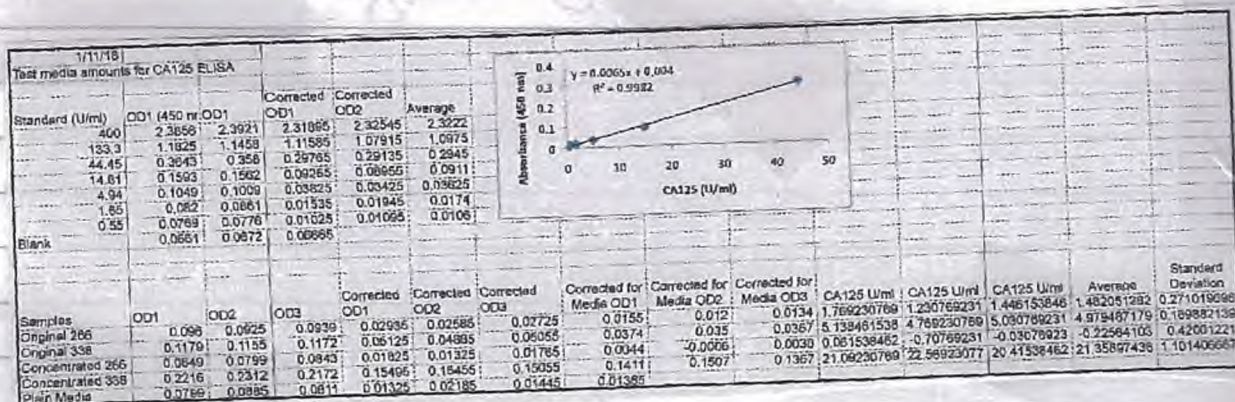
	1	2	3	4	5	6	7	8	9	10	11	12
A	Stand	A		Stand	I							
B		B		Blank								
C		C		266 orig								
D		D		338 orig								
E		E		266 conc.								
F		F		338 conc.								
G		G										
H		H										



1/11/18

CA125 ELISA - test levels in media

	1	2	3	4	5	6	7	8	9	10	11	12
A	Stand	1		266 orig								
B		2		338 orig								
C		3		266 conc								
D		4		338 conc								
E		5		media blank								
F		6										
G		7										
H	Blank											



266 = TCV120 unit

338 = Normal Ov. Epi cells

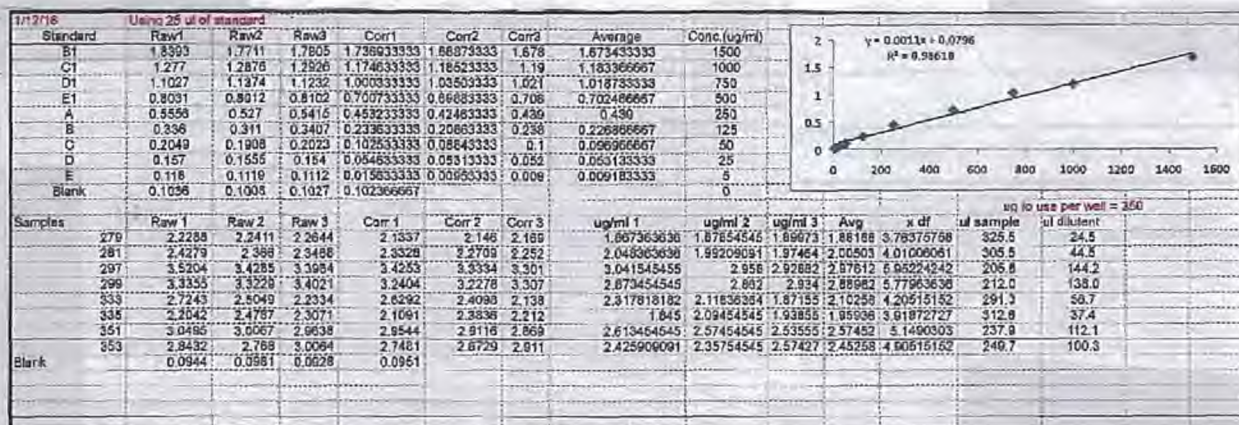
Proceed using unconcentrated media

1/12/18

Protein levels for CA125 assay

Re-did standard and original media - media was too concentrated

- diluted media by 50%, remeasured
- Also used 25 μ l of the standard and samples



1/12/2018

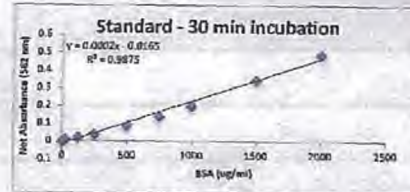
Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
TOV112D 72 hr control	279	0.104	0.0885	0.0869	0.03735	0.02185	0.03025	5.13076923	2.74615385	4.03846154	4.59451538
TOV112D 72 hr 1000 ug/ml Talc	281	0.0806	0.0742	0.0838	0.01305	0.00755	0.01715	1.63078923	0.54615385	2.02307692	1.77692308
Ov90 72 hr control	297	0.1059	0.1046	0.1321	0.03925	0.03795	0.08545	5.42307692	6.22307692	9.45384615	5.32307692
Ov90 72 hr 1000 ug/ml Talc	299	0.1062	0.0905	0.1316	0.03955	0.02395	0.09405	5.46023077	3.06923077	9.37692308	4.26923077
TOV-21G 72 hr control	333	0.0724	0.0716	0.0942	0.00675	0.00495	0.02765	0.26923077	0.14615385	3.62307692	0.20789231
TOV-21G 72 hr 1000 ug/ml Talc	335	0.0781	0.077	0.0856	0.00045	0.01035	0.01885	0.83846154	0.97892308	2.3	0.90709231
Normal Ov Epithelial 72 hr control	351	0.1003	0.0943	0.1001	0.03365	0.02785	0.03345	4.68153846	3.53846154	4.53076923	4.54615385
Normal Ov Epithelial 72 hr 1000 ug/ml Talc	353	0.1106	0.092	0.1331	0.04395	0.02535	0.09545	6.14815385	3.28461538	9.60769231	4.71538462
		0.0661	0.0672		0.06695						2.02341325

The other proteins in media may be interfering. Try lysate.

Protein levels in lysate

1-16-18

Standard ID	Concentration n (ug/ml)	OD1	OD2	OD3	Average	Corrected Avg
A	2000	0.5868	0.5848	0.5582	0.57596667	0.4874
B	1500	0.4487	0.4211	0.3533	0.4354	0.34453333
C	1000	0.3233	0.2774	0.2583	0.2868	0.19743333
D	750	0.2473	0.2199	0.2128	0.22606667	0.1391
E	500	0.181	0.1759	0.1713	0.17606667	0.0875
F	250	0.1328	0.1252	0.1256	0.12766667	0.0363
G	125	0.1105	0.112	0.1116	0.11136667	0.0228
H	25	0.1004	0.0941	0.0953	0.0966	0.00803333
I	5	0.0545	0.0540	0.0941	0.08553333	0
J (BLANK)	0	0.0551	0.0584	0.0582	0.05556667	0



	ID	OD1	OD2	OD3	Corr OD1	Corr OD2	Corr OD3	ug/ml 1	ug/ml 2	ug/ml 3	Average	using 100 ug per well	x 3.5 wells	diluent
TOV1120 72 hr control	279	0.2429	0.2301	0.2325	0.1356	0.1228	0.1263	7.806	6.965	7.09	7.0275	14.23	49.8	300.2
TOV1120 72 hr 1000 ug/ml Taic	281	0.1862	0.1523	0.1878	0.0819	0.085	0.0905	4.52	5.075	4.85	4.94833333	20.21	70.7	279.3
OV90 72 hr control	297	0.3188	0.331	0.3308	0.2125	0.2237	0.2233	11.45	12.01	11.88	12	9.33	29.2	320.5
OV90 72 hr 1000 ug/ml Taic	298	0.2629	0.2759	0.2794	0.1686	0.1711	0.1698	8.905	9.255	9.38	9.3175	10.73	27.6	312.4
TOV-21G 72 hr control	333	0.2991	0.2968	0.2949	0.1915	0.1895	0.1905	10.415	10.3	10.2	10.305	8.70	34.0	316.0
TOV-21G 72 hr 1000 ug/ml Taic	335	0.1688	0.1616	0.1634	0.0645	0.0543	0.0591	3.75	3.54	3.65	3.64	27.17	95.2	253.8
Normal Ov Epithelial 72 hr control	351	0.2359	0.23	0.23	0.1287	0.1227	0.1257	7.255	6.58	6.96	6.96	14.37	50.3	299.7
Normal Ov Epithelial 72 hr 1000 ug/ml Taic	353	0.2432	0.2474	0.2344	0.1356	0.1401	0.1271	7.82	7.83	7.18	7.25	12.94	45.3	304.7
	b	0.1097	0.1049	0.1073										

	ID	OD1	OD2	OD3	Corr OD1	Corr OD2	Corr OD3	ug/ml 1	ug/ml 2	ug/ml 3	Average	using 100 ug per well	x 3.5 wells	diluent
TOV1120 72 hr unt	278	0.0686	0.0325	0.053	0.05406667	0.03876667	0.04626667	29.063333	30.263333	29.283333	29.275333	3.42	12.0	335.0
OV90 72 hr unt 72 hr	296	0.34	0.3407	0.3306	0.23626667	0.23696667	0.23666667	12.538333	12.573333	12.468333	12.593333	7.94	27.5	322.2
TOV-121G unt	332	0.4382	0.4496	0.4415	0.33546667	0.34186667	0.33766667	17.598333	17.918333	17.703333	17.810333	5.61	19.7	330.3
Normal Ov Epithelial 72 hr unt	350	0.2281	0.2347	0.2316	0.12436667	0.13056667	0.12746667	7.043333	7.373333	7.218333	7.255333	13.71	48.0	302.0
	b	0.1032	0.104	0.104	0.10473333									

	1	2	3	4	5	6	7	8	9	10	11	12
A											279	
B											281	
C											297	
D											299	
E											333	
F	BLANK										335	
G									BLANK		351	
H											353	

	1	2	3	4	5	6	7	8	9	10
A	Stand A				I					
B	B				PBS Blank					
C	C				278					
D	D				296					
E	E				332					
F	F				350					
G	G				Lysis Buffer Blank					
H	H									

CA125 in lysate

	1	2	3	4	5	6	7	8	9	10	11	12
A	279											
B	281											
C	297											
D	299											
E	333											
F	335											
G	351											
H	353											

	1	2	3	4	5	6	7	8	9	10	11	12
A											278	
B											278	
C											296	
D											296	
E											332	
F											332	B
G											350	332
H											350	350

	Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
TOV112D 72 hr control	279	0.1637	0.1749	0.171	0.0907	0.0619	0.088	11.8	13.5230769	12.6230769	13.2230769	0.42426407
TOV112D 72 hr 1000 ug/ml Talc	281	0.1185	0.1175	0.1221	0.0358	0.0345	0.0391	4.89230769	4.89230769	4.89230769	4.89230769	0.36482413
Ov90 72 hr control	297	0.146	0.1479	0.1889	0.063	0.0649	0.0699	9.07692308	9.07692308	9.07692308	9.07692308	0.20669275
Ov90 72 hr 1000 ug/ml Talc	299	0.1371	0.13	0.1506	0.0541	0.047	0.0675	7.70769231	6.61538462	9.78461538	7.10153846	0.77237818
TOV-21G 72 hr control	333	0.1374	0.1269	0.1269	0.0544	0.0439	0.0459	7.75384615	6.13846154	6.44615385	6.29230769	0.21757132
TOV-21G 72 hr 1000 ug/ml Talc	335	0.1	0.0899	0.0907	0.017	0.0098	0.0077	2	0.29230769	0.56923077	0.43076923	0.19581419
Normal Ov Epithelial 72 hr control	351	0.1491	0.1518	0.1547	0.0991	0.0686	0.0717	9.55384615	9.90623077	10.4153846	9.76153846	0.29372128
Normal Ov Epithelial 72 hr 1000 ug/ml talc	353	0.1551	0.1302	0.1467	0.0721	0.0532	0.0637	10.4769231	7.98923077	9.18461538	9.83076923	0.91378953

	Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
TOV112D 72 hr unit	278	0.1998	0.1904	0	0.0476	0.0382	0.0468	8.70769231	5.26153846	5.98461538	6.31538462	1.02258519
Ov90 72 hr unit 72 hr	296	0.1976	0.1871	0.199	0.0456	0.0440	0.0488	4.81538462	3.60769231	6.58461538	4.10153846	0.07814996
TOV-121G unit	332	0.1875	0.179	0.2328	0.0353	0.0286	0.0605	14.9384615	20.1846154	11.7846154	13.3615385	0.0245781
Normal Ov Epithelial 72 hr unit	350	0.2533	0.2874	0.1522	0.1011	0.1352						2.230106

Results: Lysate protein measurements may be affected by talc.
Repeat protein measurements, have control w/ talc in it.

Remeasured protein levels in media ⁴¹⁰⁴⁷

° Recalc. data. CA125

1-19-18

	1	2	3	4	5	6	7	8	9	10	11	12
A				BLANK		279						
B				BLANK		281						
C				BLANK		297						
D						299						
E				BLANK		333						
F						335						
G						351						
H						353						

Re-measure media protein

1-19-18

- 10x diluted samples
- media was 10x as blank
- used 25ul to detect

	1	2	3	4	5	6	7	8	9	10	11	12
A							278			335		
B							279			350		
C							281			353		
D							296			353		
E							297			Lysis Buffer		
F							299			Lysis Buffer + talc		
G							332					
H							333					

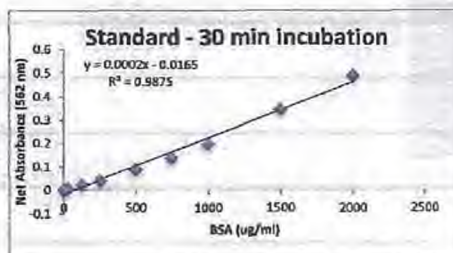
protein → New control

$$44 \times 200 = \frac{8800}{50} = 176$$

Go to pg 17

from pg 16

Standard ID	Concentration (ug/ml)	OD1	OD2	OD3	Average	Corrected Avg
A	2000	0.5869	0.5848	0.5562	0.575967	0.4874
B	1500	0.4457	0.4211	0.3533	0.4334	0.344833
C	1000	0.3213	0.2774	0.2593	0.286	0.197433
D	750	0.2473	0.2199	0.2128	0.226667	0.1381
E	500	0.181	0.1759	0.1713	0.176067	0.0875
F	250	0.1328	0.1252	0.1258	0.127667	0.0393
G	125	0.1105	0.112	0.1118	0.111367	0.0228
H	25	0.1004	0.0941	0.0953	0.0966	0.008033
I	5	0.0845	0.0846	0.0941	0.087733	0
J (BLANK)	0	0.0881	0.0884	0.0892	0.088567	0



	ID	OD1	OD2	OD3	Corr OD1	Corr OD2	Corr OD3	ug/ul 1	ug/ul 2	ug/ul 3	Average	how much was used	ug used
TOV112D 72 hr unt	278	0.6562	0.6792	0.6667	0.559667	0.6792	0.6667	28.81833	34.785	34.16	34.4725	3.42	117.9
TOV112D 72 hr control	279	0.2121	0.2206	0.2113	0.102767	0.2206	0.2113	5.963333	11.855	11.99	11.6225	14.23	165.4
TOV112D 72 hr 1000 ug/ml Talc	281	0.1868	0.1902	0.189	0.077267	0.1902	0.189	4.688333	10.335	10.275	8.432778	20.21	170.4
Ov90 72 hr unt 72 hr	298	0.3372	0.3405	0.3354	0.227867	0.3405	0.3354	12.21833	17.85	17.595	15.88778	7.94	126.1
Ov90 72 hr control	297	0.3217	0.3231	0.3255	0.212367	0.3231	0.3255	11.44333	16.98	17.1	17.04	8.33	141.9
Ov90 72 hr 1000 ug/ml Talc	299	0.2813	0.282	0.2785	0.171967	0.282	0.2785	9.423333	14.925	14.75	14.8375	10.73	158.2
TOV-121G unt	332	0.4161	0.4363	0.452	0.306767	0.4363	0.452	16.16333	22.79	23.425	23.1075	5.61	129.6
TOV-121G 72 hr control	333	0.3031	0.3039	0.3238	0.193767	0.3039	0.3238	10.51333	16.02	17.015	14.51611	9.70	140.8
TOV-121G 72 hr 1000 ug/ml Talc	335	0.1847	0.1856	0.18	0.075367	0.1856	0.18	4.563333	10.105	10.325	8.541111	27.47	228.1
Normal Ov Epithelial 72 hr unt	360	0.2227	0.2338	0.2367	0.113367	0.2338	0.2367	6.493333	12.515	12.66	12.5875	13.71	172.6
Normal Ov Epithelial 72 hr control	351	0.2287	0.2316	0.2402	0.119367	0.2316	0.2402	6.793333	12.405	12.835	12.62	14.37	181.3
Normal Ov Epithelial 72 hr 1000 ug/ml Talc	353	0.2277	0.2391	0.2432	0.118367	0.2391	0.2432	6.743333	12.78	12.985	9.761667	12.94	126.3
Lysis buffer blank		0.1102	0.1063	0.1095	0.109333								
Lysis buffer+talc blank		0.1073	0.1068	0.1059	-0.00203	-0.00253	-0.00343	-0.00267					

Remeasured Media Protein - 10x diluted using 25ul standard

Samples	Raw 1	Raw 2	Raw 3	Corr 1	Corr 2	Corr 3	ug/ul 1	ug/ml 2	ug/ml 3	Avg	xdif	ul media used	actual ug used
279	0.7155	0.7669	0.7571	0.6192	0.6608	0.6908	0.490545	0.528182	0.526364	0.515697	5.15897	92.992174	479.5678
281	0.7067	0.7199	0.7356	0.6104	0.6176	0.6393	0.482545	0.489091	0.508818	0.493485	4.93485	87.280476	430.7159
297	1.1257	1.1415	1.1856	1.0294	1.0455	1.0963	0.853455	0.878091	0.899727	0.880424	8.80424	58.801368	517.7015
299	1.1303	1.1325	1.1842	1.034	1.0562	1.0879	0.887636	0.886636	0.916636	0.886636	8.86636	60.557443	535.7132
333	0.7006	0.7217	0.7401	0.6043	0.6254	0.6438	0.477	0.496182	0.512909	0.495364	4.95364	83.231246	412.2973
335	0.7315	0.7361	0.7684	0.6352	0.6398	0.6721	0.505091	0.508273	0.538836	0.517667	5.176667	89.314713	462.3525
351	0.9217	0.9433	0.9655	0.8254	0.847	0.8592	0.578	0.597636	0.717818	0.697616	6.97616	67.973964	474.3347
353	0.9401	0.9952	1.0083	0.8438	0.8689	0.912	0.694727	0.744818	0.756727	0.732091	7.320909	71.353555	522.3729
Blank-10x media	0.7339	0.7117	0.7024	0.6376	0.7117	0.7024	0.70706						
Blank-PBS	0.0941	0.0998	0.095	0.0963									

Recalculated how much protein was really used
and then adjusted CA125 levels pg 18

from ps 17

CA125-recalc. for SKI abstract

1/12/2018																		
		Sample	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 Unit	CA125 Unit	CA125 Unit	Average	Standard Deviation	Corrected per up protein	Corrected per up protein	Corrected per up protein	Average	SD
MEDIA		279	0.114	0.080	0.088	0.0773	0.0519	0.0528	5.1079325	2.7491835	4.03649154	4.55481533	0.77237316	0.01059893	0.0077543	0.00942122	0.28787	0.00191
TOV1120 72 hr control		281	0.0026	0.0743	0.0836	0.0135	0.0755	0.0719	1.0017032	5.5481838	2.02207327	1.77852664	0.34811411	0.00193294	0.00115587	0.00421663	0.00071	0.00077
TOV1120 72 hr control		292	0.1058	0.1048	0.1321	0.0923	0.0796	0.0954	5.42370802	5.22357992	9.43264815	5.32878932	0.14142136	0.01130548	0.0106144	0.01371367	0.01110	0.00029
OV90 72 hr control		298	0.1052	0.0905	0.1318	0.0915	0.0836	0.0948	5.48929077	3.06923777	9.37852358	4.36819177	1.89798027	0.01140474	0.00845013	0.01855327	0.01548	0.00075
Cov02 72 hr 1000 upstnl Talc		333	0.1254	0.0719	0.0942	0.0375	0.0649	0.0275	0.28120777	0.14145369	0.02574922	0.26790231	0.05702853	0.00555141	0.00054977	0.00155224	0.00019	0.00019
TOV-21G 72 hr control		335	0.0781	0.077	0.0908	0.0249	0.0185	0.0185	0.83840154	0.97692308	-	2.3	0.90718031	0.00790788	0.00174341	0.00476528	0.00189	0.00023
TOV-21G 72 hr 1000 upstnl Talc		351	0.1053	0.0943	0.1001	0.0336	0.0269	0.0269	4.59192840	3.82848154	4.32078333	4.5481838	0.02179713	0.00511197	0.00758712	0.00644781	0.00048	0.00055
Normal Ov Epithelial 72 hr control		353	0.1108	0.082	0.1301	0.0400	0.0335	0.0515	5.19170385	0.28491308	0.90791231	4.71328462	2.5311325	0.01581629	0.00094228	0.0003448	0.01543	0.00010
Normal Ov Epithelial 72 hr 1000 upstnl Talc		355	0.0901	0.0872	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		3	0.2081	0.052	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1/12/2018																		
		Sample	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 Unit	CA125 Unit	CA125 Unit	Average	Standard Deviation	Corrected per up protein	Corrected per up protein	Corrected per up protein	Average	SD
TOV1120 72 hr control		278	0.1968	0.1904	0.171	0.0478	0.0382	0.0382	6.70758291	6.29105848	3.35401328	1.02255118	0.20666052	0.04452688	-	0.0059	0.00017	
TOV1120 72 hr control		279	0.1637	0.1749	0.171	0.0687	0.0919	0.0919	11.8	12.322758	12.322758	13.2230769	6.42495477	0.0712473	0.0817088	0.07813736	0.0609	0.00026
TOV1120 72 hr 1000 upstnl Talc		281	0.1188	0.1175	0.1221	0.0238	0.0545	0.0545	0.0301	4.89227099	4.89227099	5.44048719	0.36151113	0.00760227	0.00750275	0.00199322	0.00183	0.00021
OV90 72 hr control		292	0.1878	0.1971	0.1971	0.0453	0.0448	0.0448	6.4	6.2022758	6.2022758	6.34114085	0.07614079	0.00772387	0.0448788	-	0.0006	0.00026
Cov02 72 hr 1000 upstnl Talc		297	0.146	0.1478	0.1478	0.053	0.0549	0.0549	0.0009	0.0002008	0.00023177	12.8	0.23337062	0.00088775	0.0006669	0.00087879	0.00030	0.00018
TOV-21G control		333	0.1371	0.13	0.1396	0.0541	0.047	0.047	0.0676	7.75182021	0.81525462	0.70461538	7.10120445	0.7737816	0.04841321	0.04185235	0.06146569	0.01291
TOV-21G 72 hr control		335	0.1575	0.178	0.180	0.0597	0.0528	0.0528	0.0458	4.51233402	3.50783251	6.56461328	4.18125045	0.8246701	0.03714877	0.02704912	0.03970425	0.00171
TOV-21G 72 hr 1000 upstnl Talc		351	0.1374	0.1569	0.1298	0.0544	0.0438	0.0438	0.0408	7.75334915	0.13045154	0.44613285	0.28330769	0.2777132	0.05080747	0.04305928	0.0403853	0.00159
Normal Ov Epithelial 72 hr control		353	0.1	0.0889	0.0807	0.017	0.0201	0.0201	0.0877	2.26239708	0.05825677	6.42078125	0.19551418	0.05872886	0.00117173	0.00248431	0.00042	0.00048
Normal Ov Epithelial 72 hr 1000 upstnl Talc		355	0.2233	0.2054	0.2208	0.1071	0.1382	0.1382	0.1886	14.9354915	20.1646154	11.7349154	12.2611335	0.05082829	0.11869186	0.00023287	0.00774	0.00129
Normal Ov Epithelial 72 hr control		357	0.1491	0.1516	0.1547	0.0008	0.0088	0.0088	0.0717	0.55354615	0.96833077	10.1153848	0.78182848	0.29372128	0.0258108	0.0647235	0.02743689	0.00016
Normal Ov Epithelial 72 hr 1000 upstnl Talc		359	0.1391	0.1352	0.1467	0.0224	0.0332	0.0332	0.0537	10.4798231	7.58823877	0.18451533	0.83070823	0.91379952	0.07726881	0.00052239	0.0272144	0.00020
Normal Ov Epithelial 72 hr 1000 upstnl Talc		360	0.0988	0.0878	0.0748	0.005	-	-	-	-	-	-	-	-	-	-	-	-

fold increase fold increase fold increase Average SD

OV90	1.02745665	0.5765896	1.76156069	1.39450867	0.51908995
TOV21G	4.03703704	4.7037037	11.0740741	4.37037037	0.47140452
Normal Ov Epi	1.35194585	0.72250423	2.11336717	1.73265651	0.53840618

Media calculations for OV90, 21G, Normal Ov. Epi were used for SKI abstract

1/29/18

Seed 72 hour 1000 $\mu\text{g/ml}$ talc EOC

SKW-3 and A2780 seeded 4×10^6 cells

TAV-21G seeded 2×10^6 cells

Treat SKW-3 and A2780 today 1/29/18
25ml of media

$$(x)(100 \text{ mg/ml}) = (25 \text{ ml})(1000 \text{ } \mu\text{g/ml})$$

$$x = 250 \text{ ml}$$

Treat TAV21G 1/30/18

1/31/18 - The presence of 1000 $\mu\text{g/ml}$ is physically killing the cells.
- We need to decrease dose.

4/3 2/1/18

Treat EOC w/ talc

- Needed to lower the dose of talc (in saline) ^{PBS} → 72 hours
- unt, 5, 20, 100 $\mu\text{g/ml}$

- Seeded 1×10^6 cells ID

60mm dish, 5ml

- ELI unt 356

ELI 5 $\mu\text{g/ml}$ 357

ELI 20 $\mu\text{g/ml}$ 358

ELI 100 $\mu\text{g/ml}$ 359

- SKOV-3 unt 360

5 $\mu\text{g/ml}$ 361

20 $\mu\text{g/ml}$ 362

100 $\mu\text{g/ml}$ 363

- TON1120 unt 364

5 $\mu\text{g/ml}$ 365

20 $\mu\text{g/ml}$ 366

100 $\mu\text{g/ml}$ 367

- A2780 unt 368

5 $\mu\text{g/ml}$ 369

20 $\mu\text{g/ml}$ 370

100 $\mu\text{g/ml}$ 371

- OVR90 unt 372

5 $\mu\text{g/ml}$ 373

20 $\mu\text{g/ml}$ 374

100 $\mu\text{g/ml}$ 375

FT 33

No treatment unt 379

5 $\mu\text{g/ml}$ 380

20 381

100 382

1000 383

Control 5 377

Control 1000 378

ELI Control-5 379

Control-1000 380

1000 $\mu\text{g/ml}$ talc 381

SKOV-3 Control 5 382

Control 1000 383

1000 $\mu\text{g/ml}$ talc 384

A2780 Control-5 385

Control 1000 386

1000 $\mu\text{g/ml}$ talc 387

Normal A/Epi - unt 388

5 5 $\mu\text{g/ml}$ 389

20 20 390

100 100 391

1000 392

Control 5 393

Control 1000 394

TON1120 Control 5 395

Control 1000 396

1000 $\mu\text{g/ml}$ talc 397

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Prepare talc

100 mg in 10 mL \rightarrow mix and filter = 10 mg/mL

2/2/18 treated w/ talc

$$(x)(10000 \text{ ug/mL}) = (5 \text{ mL})(5 \text{ ug/mL}) = 2.5 \text{ mL}$$

$$(x)(10000 \text{ ug/mL}) = (5 \text{ mL})(20 \text{ ug/mL}) = 10 \text{ mL}$$

$$(x)(10000 \text{ ug/mL}) = (5 \text{ mL})(100 \text{ ug/mL}) = 50 \text{ mL}$$

2/6/18

treat w/ "soaked" talc (10000 ug/mL)

"talc was rocked for 72 hours, spun and supernatant collected"

★ too much volume

made 1g/10 mL DMSO - Re-soak 72 hrs

2/11/18 seeded Normal Ov. Epi Cells $1 \times 10^6 \rightarrow$ treat Friday 2/18/18 ✓

2/26/18

Monday 2/26/18

383 NOE

0 ug/mL	383
5 ug/mL	384
20 ug/mL	385
100 ug/mL	386

2/5/18

RNA

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	356	2/5/2018 1:18:50 PM	0.0830	µg/µl	2.074	1.109	1.87	1.30	RNA	40.00
2	357	2/5/2018 1:19:20 PM	0.1000	µg/µl	2.500	1.342	1.86	1.18	RNA	40.00
3	358	2/5/2018 1:19:39 PM	0.0829	µg/µl	2.073	1.118	1.85	1.26	RNA	40.00
4	359	2/5/2018 1:20:00 PM	0.0349	µg/µl	0.873	0.476	1.84	0.39	RNA	40.00
5	360	2/5/2018 1:20:24 PM	0.2387	µg/µl	5.968	2.966	2.01	0.78	RNA	40.00
6	361	2/5/2018 1:20:43 PM	0.3389	µg/µl	8.473	4.194	2.02	1.15	RNA	40.00
7	362	2/5/2018 1:21:04 PM	0.3017	µg/µl	7.542	3.837	1.97	1.47	RNA	40.00
8	363	2/5/2018 1:21:20 PM	0.1118	µg/µl	2.796	1.465	1.91	1.53	RNA	40.00
9	368	2/5/2018 1:21:41 PM	0.2203	µg/µl	5.508	2.880	1.91	1.34	RNA	40.00
10	369	2/5/2018 1:21:57 PM	0.2474	µg/µl	6.185	3.187	1.94	2.03	RNA	40.00
11	370	2/5/2018 1:22:12 PM	0.2217	µg/µl	5.541	2.855	1.94	1.63	RNA	40.00
12	371	2/5/2018 1:22:29 PM	0.1336	µg/µl	3.340	1.726	1.93	1.42	RNA	40.00

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	379	2/16/2018 9:27:37 AM	0.1685	µg/µl	4.212	2.034	2.07	1.01	RNA	40.00
2	380	2/16/2018 9:27:55 AM	0.0658	µg/µl	1.645	0.713	2.31	3.02	RNA	40.00
3	381	2/16/2018 9:28:13 AM	0.0801	µg/µl	2.003	0.891	2.25	0.96	RNA	40.00
4	382	2/16/2018 9:28:30 AM	0.3084	µg/µl	7.711	3.759	2.05	2.24	RNA	40.00
5	383	2/16/2018 9:28:51 AM	0.2921	µg/µl	7.303	3.582	2.04	1.09	RNA	40.00
6	384	2/16/2018 9:29:10 AM	0.1812	µg/µl	4.531	2.179	2.08	2.15	RNA	40.00
7	385	2/16/2018 9:29:29 AM	0.0869	µg/µl	2.172	0.971	2.24	1.31	RNA	40.00
8	386	2/16/2018 9:29:51 AM	0.0116	µg/µl	0.289	-0.017	-16.61	-5.65	RNA	40.00
9	387	2/16/2018 9:30:11 AM	0.0133	µg/µl	0.332	0.013	25.53	-15.74	RNA	40.00
10	395	2/16/2018 9:30:29 AM	0.2169	µg/µl	5.421	2.598	2.09	0.65	RNA	40.00
11	397	2/16/2018 9:30:51 AM	0.1328	µg/µl	3.321	1.575	2.11	2.26	RNA	40.00
12	396	2/16/2018 9:31:08 AM	0.1633	µg/µl	4.084	1.949	2.10	2.47	RNA	40.00

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
16	364	2/16/2018 9:49:26 AM	0.2401	µg/µl	6.003	2.879	2.09	1.78	RNA	40.00
17	365	2/16/2018 9:49:46 AM	0.2418	µg/µl	6.044	2.939	2.06	1.27	RNA	40.00
18	366	2/16/2018 9:50:01 AM	0.2043	µg/µl	5.106	2.459	2.08	1.88	RNA	40.00
19	367	2/16/2018 9:50:16 AM	0.1712	µg/µl	4.281	2.026	2.11	1.83	RNA	40.00

41054

CDNA

- supers VILO
Kit

2/16/18

0.5 ug RNA Reaction

ID	ul RNA	ul water
356	6.0	10.0
357	5.0	11.0
358	6.0	10.0
359	14.3	1.7
360	2.1	13.9
361	1.5	14.5
362	1.7	14.3
363	4.5	11.5
364	2.1	13.9
365	2.1	13.9
366	2.4	13.6
367	2.9	13.1
368	2.3	13.7
369	2.0	14.0
370	2.3	13.7
371	3.7	12.3
379	3.0	13.0
380	7.6	8.4
381	6.2	9.8
382	1.6	14.4
383	1.7	14.3
384	2.8	13.2
385	5.8	10.2
386	16.0	0.0
387	16.0	0.0
395	2.3	13.7
397	3.8	12.2
396	3.1	12.9

